Original Article

A Saliva's Physicochemical Characteristics And Total Salivary Antioxidants Ex Vivo Research Evaluating The Effect Of Probiotics

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Abstract

BACKGROUND: It has long been proven that oxidative stress plays a role in the pathogenesis of several illnesses. In addition to acting as free radical scavengers and preventing cell damage, antioxidants also have an impact on the metabolism of a number of pathogenic bacteria. Alternative therapeutic treatment alternatives are becoming more prevalent as antibiotic resistance rates rise. Probiotics, according to the WHO, are the second-most important immune defense mechanism after antibiotic resistance. Probiotics' specific mode of action is still up for debate, despite several research emphasizing their beneficial effects on illnesses. The goal of the current research was to reveal the probiotics' antioxidant properties in saliva as well as how they affected the pH and buffering power of the saliva.
METHODS: The study was conducted in PAC Hospital Kamra. Before and after consuming probiotics for two weeks, unstimulated saliva from 10 people was tested for total antioxidant content, pH, and buffering capacity using a spectrophotometer, pH paper, and buffer strips, respectively. For this research, healthy adults between the ages of 20 and 35 were included; those who often smoked or drank alcohol as well as those with any systemic diseases were excluded. After 14 days of probiotic use, the whole procedure for determining the pH, buffering capacity, and total antioxidant content in saliva was carried out, and comparisons between the three parameters were made.
RESULTS: To check for changes, the total antioxidant content, pH, and buffering capacity were tested before and after for the whole sample. While pH and buffering capacity showed negligible findings with p-values of 1.00 and 0.08, respectively, total antioxidant level demonstrated significant outcomes with a p-value of 0.003. The overall findings demonstrated that probiotic consumption increased the amount of total salivary antioxidants without significantly affecting pH or buffering capacity. The "paired t-test" was used to statistically assess the results.
CONCLUSIONS: Probiotics are useful for boosting antioxidant levels, which reduces cellular damage and makes them less likely to cause illness. Additionally, an increase in antioxidant levels may be readily detected in saliva, making it a useful diagnostic tool.

KEYWORDS: saliva, probiotics, antioxidant level.

INTRODUCTION:

An imbalance between the quantities of pro- and antioxidants in the cell leads to the homeostatic phenomena known as oxidative stress. (1) As a result of this imbalance, free radicals are created, which later cause DNA hydroxylation, protein denaturation, lipid peroxidation, and cell death. (2) An excessive quantity of these reactive radicals impairs cell viability and is a factor in a number of disorders, including those that affect the mouth, such as oral precancerous lesions, periodontitis, and dental caries. (3) With the spread of illness over the last several

years and the understanding that oxidative stress plays a significant role in their pathogenesis, the antioxidant modality has been the focus of our preventative and therapeutic efforts. (4) Bacteriotherapy utilizing probiotics is one of these cutting-edge methods. Probiotics are living non-pathogenic bacteria that, when given in sufficient quantities, have positive effects on health. (5) This time-tested idea of bacteriotherapy is renowned for its positive outcomes and is thought to help minimize harm by lowering oxidative stress. (6) The precise process hasn't yet been figured out. Probiotic microorganisms have considerable antioxidant properties both in vitro and in vivo, according to evidence. However, the clinical trials looked at probiotics' antioxidant capacity in plasma (7–10). Since the extremely contentious Vipeholm research, which was done in the 1950s, it has been recognized that frequent carbohydrate ingestion has negative long-term clinical effects (11,12). Less is known about the immediate effects of sugar stress on oral homeostasis, and there aren't any well-powered in vivo studies describing how excessive carbohydrate consumption affects the makeup of the oral microbiota in otherwise healthy people. In reality, saliva, which can be retrieved quickly and painlessly, shows how the oral microbiota is made up and includes useful inflammatory markers. (13,14) Importantly, research has shown that the composition of the salivary microbiota and salivary levels of inflammatory markers not only reflect the state of oral health (15,16), but are also susceptible to outside stressors such poor diet, smoking, and dental hygiene. (17,18,19) In order to study the impact of coordinated perturbations on oral homeostasis, analysis of salivary microbiota and inflammatory marker levels provides a wonderful model system. However, to our knowledge, this model system has never been used to evaluate the short-term effects of frequent carbohydrate consumption on oral homeostasis and to ascertain whether concomitant probiotic administration has any protective effects when dental homeostasis is hampered by frequent sugar intake.

The use of saliva as a diagnostic technique has a number of benefits over plasma. It is the best method for screening, diagnosing, and monitoring a variety of illnesses since it is readily available, simple to collect, allows for repeated non-invasive samples, requires a simpler procedure, and provides results more quickly. (20) The pathophysiology of oxidative stress in saliva has been the subject of a very small number of research, and probiotics' possible influence on salivary antioxidant levels has never been investigated. Additionally, it is unclear how probiotics affect the pH and salivary buffering capacity. Thus, the study's objective was to assess how probiotics affected the amount of total salivary antioxidants and the physical characteristics of saliva (pH and buffering capacity).

RESOURCES AND METHODS:

The study was conducted in PAC Hospital Kamra. Among the tools used in this experiment were a sterile saliva bottle, Yakult probiotic drink, glass tube, pipettes, centrifuge, buffering strips, pH strips (GC Saliva Check Kit), hot water bath, and spectrophotometer.

The study was carried out in September over a two-week period after receiving institutional ethical approval. The research only included people who agreed to participate willingly and knowingly. Healthy people between the ages of 20 and 35 were recruited for this study; those who smoked or drank often were excluded, as did those with any systemic disorders. Subjects were told not to eat or drink anything for at least an hour before saliva collection on the study day. To minimize circadian variations, samples were collected between 9 and 10 in the morning.

Subjects were told to collect the accumulated saliva into sterile saliva vials after saliva was allowed to build on the mouth's floor (Passive drool method). After collection, samples were delivered to the lab in less than 30 minutes. Within an hour, salivary samples were analyzed for pH, buffering ability, and total antioxidant content. Then, for the following two weeks, subjects received one serving of Yakult, a probiotic beverage.

Table 1: Before and after probiotic delivery, buffering capacity, pH, and total salivary antioxidant content

	Total antioxidant capacity		**Buffering capacity**		**pH**	
Sample	**Before**	**After**	**Before**	**After**	**Before**	**After**
1	0.869	1.328	4	4	7.2	7.4
2	1.124	1.435	3	3	7.2	7.2

3	1.097	1.269	4	4	6.8	6.8
4	0.983	1.225	4	4	7.2	7.4
5	0.809	0.783	4	4	7	6.8
6	0.71	1.377	4	3	7.4	7.2
7	0.814	1.002	3	3	7.6	7.6
8	0.746	1.224	4	4	7.4	7.2
9	0.988	1.492	4	3	6.8	6.8
10	1.283	1.257	4	4	7	7.2

After 14 days of probiotic consumption, the same technique for measuring salivary total antioxidant level, pH, and buffering capacity was performed, and comparisons were conducted between the three parameters. A test tube containing 100 microliters of saliva and 5% trichloroacetic acid was used. After 5 minutes of settling, it underwent 10 minutes of centrifugation. 100 microliters of the supernatant saliva were mixed with 1 mL of the total antioxidant reagent. For 90 minutes, this combination was incubated at 900C in a hot water bath. A spectrophotometer was used to read the optical density at 695 nm. Estimation of buffering capacity and pH: Buffering strips were coated with an adequate quantity of saliva, and a noticeable color shift was seen. The leftover spit in the vial was applied on pH paper, and a color shift was seen. The GC Salivary Check kit's color guide served as the basis for interpretation.

DATA ANALYSIS: IBM SPSS version 26 was used to statistically analyze the data that were collected. By using a "paired samples t-test," differences between variables were analyzed. Means and standard deviations were recorded so that a descriptive analysis could be performed (Table 2). Variables were displayed on a statistical graph to compare pH, buffering capacity, and total antioxidant level before and after probiotic treatment.

RESULTS: The total antioxidant content, pH, and buffering capacity were measured before and after for the whole sample to see whether any changes occurred (Table 1). A paired samples t-test was used to detect any significant changes (Table 2). With p-values of 0.003 and a t-test value of -4.08, the findings of the paired samples t-test show that there is a significant difference in the number of total antioxidants before and after probiotic treatment. Total antioxidant levels had a mean value of 0.94 with a standard deviation of 0.19 before and a mean value of 1.24 with a standard deviation of 0.21 after. The average difference in the number of total antioxidants was -0.13. With p-values of 1.00 and 0.08, respectively, the findings for pH and buffering capacity indicated no statistically significant differences. The pH T-stat value was 0, but the buffering capacity T-stat value was 1.96. The pH had a mean value of 7.16 before, with a standard deviation of 0.26, and a mean value of 7.16 after, with a standard deviation of 0.28. For pH, the average difference was 0. The buffering capacity had a mean value of 3.8 with a standard deviation of 0.42 before and a mean value of 3.5 with a standard deviation of 0.53 after. The average pH difference was 0.3.

Table 2: Statistical analysis and results of paired samples t-test with mean and standard deviation values.

					Confidence Interval (95%)				
	Different parameters (n)	**Average**	**S.D**	**Mean difference**	Lower Limit	Upper Limit	t-stat	df	prob value
Total antioxidant level	Before (10)	0.94	0.19	-0.3	-0.46	-0.13	-4.08	9	0.003
	After (10)	1.24	0.21						
pH	Before (10)	7.16	0.26	0	-0.12	0.12	0	9	1.00
	After (10)	7.16	0.28						
Buffering capacity	Before (10)	3.8	0.42	0.3	-0.05	0.65	1.96	9	0.08
	After (10)	3.5	0.53						

Consuming probiotics enhanced the number of total antioxidants in saliva while having little to no effect on pH or buffering capacity, according to the findings of the paired samples t-test. The pH of the saliva in the current investigation did not change as a result of probiotic administration (Figure 1). After consuming probiotics for two weeks, there was no change in the saliva's capacity to serve as a buffer (Figure 2). Not least of all, probiotic supplementation raised the content of all antioxidants in saliva (Figure 3).

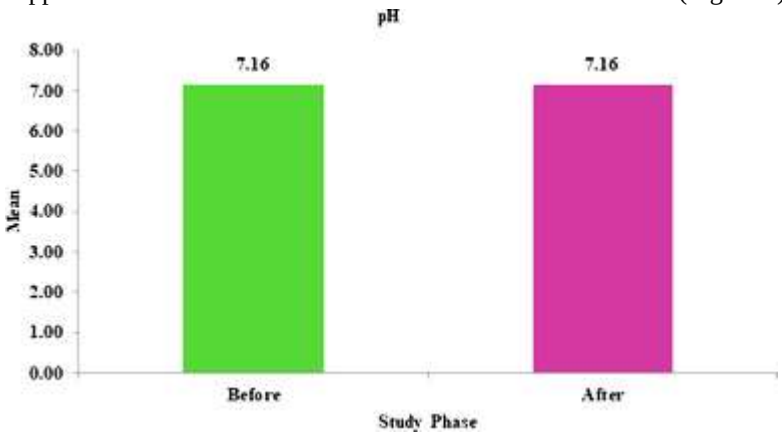

Figure 1: pH variation before and after probiotic use

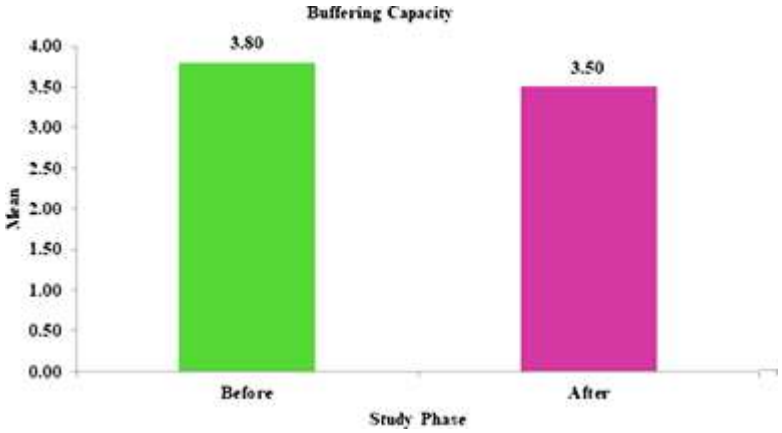

Figure 2: Pre- and post-probiotic ingestion variations in buffering capacity

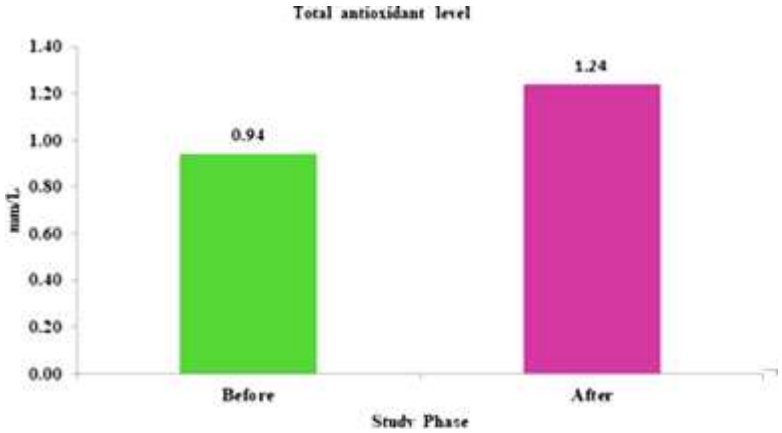

Figure 3: Probiotics' impact on the number of total antioxidants both before and after administration

DISCUSSION: Bifidobacterium and Lactobacillus are two of the most widely utilized probiotic microorganisms. Despite the controversy surrounding its usage, recent studies have shown that lactic acid bacteria have a beneficial effect on halitosis, periodontitis, dental caries, and a variety of other oral disorders. (21) There was no observable difference in the pH of the saliva after probiotics were administered in the present study. This

result is in line with other studies that found no discernible pH change after the addition of probiotic bacteria to a biofilm. The unaltered phenomenon in the present investigation may be explained in one of two ways:
a) By using the acids produced by Lactobacillus, the other microbes in the oral cavity prevented environmental impacts.
b) The pH was unaffected by the acids generated by lactobacillus cells since they made up a very small fraction of saliva.
A key host defense mechanism, saliva buffer controls the pH of the mouth environment. (23) Saliva's ability to act as a buffer remained unchanged after two weeks of probiotic use. In contrast to this result, a prior study found that probiotic treatment increased buffering capacity. (24) This result might be explained by
a) The lack of any noticeable pH shift suggests that probiotic distribution did not result in any significant ionic interactions that would have impacted buffering capacity.
b) In addition, the study was conducted for a shorter period of time than earlier studies, which typically lasted at least six months.
Last but not least, probiotic supplementation increased saliva's level of all antioxidants. This outcome is in line with a prior investigation that discovered probiotics increase plasma antioxidant levels. (25) These considerations may help to explain this result: a) Lactobacillus, a probiotic bacterium with superoxide dismutase activity, was present in the probiotic beverage utilized in the study.
b) other ways by which plasma and saliva are exchanged, resulting in the presence of biomarkers in saliva, include active and passive diffusion.

CONCLUSION:

Probiotics have been shown to maintain a healthy microbial ecology in the past. However, it also affects cells. Probiotics raise the body's number of antioxidants. These antioxidants stop the production of free radicals, halting cell harm in its tracks. Additionally, it has an impact on the metabolism of several microbial cells, particularly those in charge of oral illnesses. Saliva may also be used to detect changes in antioxidant levels after probiotics. Therefore, using saliva as a regular diagnostic technique to determine the amount of all antioxidants is possible. It may also be used to assess how probiotics affect a particular antioxidant system linked to a certain illness.

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